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Evaluation of the Possible Anti-fibrotic Effect of Rosuvastatin on bleomycin-induced pulmonary fibrosis in mice model

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Keywords

- rosuvastatin
- bleomycin-induced pulmonary fibrosis
- pirfenidone
- TGF-β

Abstract

Background: Idiopathic Pulmonary fibrosis (IPF) is a serious, progressive lung disease characterized by accumulation of extracellular matrix (ECM), leading to functional deterioration in lung parenchyma. **Objectives:** to characterize the effect of rosuvastatin on bleomycin-induced pulmonary fibrosis in mice, to compare its effect with the well-established anti-fibrotic effect of pirfenidone, and the role of transforming growth factor-βeta (TGF-β). **Materials & methods:** induction of pulmonary fibrosis was done by intra-tracheal injection of bleomycin (2U/Kg, 50 µl) to C57BL/6 mice. Then, at the 10^{th} day; pirfenidone (300mg/kg/d) or rosuvastatin (10mg/kg/d) given orally. After 21 days, all mice were sacrificed. Body weight, lung weight, lung index and lung TGF-β1were measured before and after induction of pulmonary fibrosis. Histopathological examinations of lung sections were done using haematoxylin and eosin. Also, Masson's trichrome stain was used for scoring fibrosis. **Results:** Significant decrease in body weight and increase in lung weight, lung index, TGF-β levels were observed in bleomycin group. Also, the histopathological fibrosis score was increased in comparison to control group. Both groups of pirfenidone and rosuvastatin showed significant increase in body weight, and decrease in lung weight, lung index, TGF-β levels. Histopathological fibrosis score was decreased in comparison to bleomycin group. **Conclusions:** Rosuvastatin effectively reduced pulmonary fibrosis as pirfenidone. This was done by decreasing TGF-β levels. Thus, the pleiotropic actions of rosuvastatin might be applied clinically for prevention or treatment of bleomycin-induced pulmonary fibrosis. However, further studies are needed to confirm the beneficial therapeutic effects of rosuvastatin in the therapy of bleomycin-induced pulmonary fibrosis.

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Introduction

IPF is a chronic and progressive interstitial lung disease (ILD) characterized by abnormal ECM deposition. The exact etiology is poorly understood, involving interplay of a variety of risk factors in genetically susceptible patients **(Liu and Shi, 2019)**.

IPF is manifested by progressive disease and ultimately respiratory failure and death **(Milne et al., 2018)**. Despite considerable progress in IPF medical treatment, the only effective therapy is lung transplantation, which is however burdened by significant risks of complications and death **(Comeglio et al., 2017).**

IPF is one of the most common forms of ILDs. The incidence of IPF increases dramatically with age, leading to significant burden on health care system. IPF is diagnosed by finding of radiographic and/or histopathology, so, the diagnosis of IPF is by exclusion of other causes of interstitial pneumonia **(Martinez et al., 2017).** The prognosis of IPF is very poor, with median survival range from 2 to 5 years **(Li et al., 2018).**

Pirfenidone was approved as anti-fibrotic drug in IPF. Although, it has improved IPF, as it decreases the declining of lung functions, it cannot prevent the disease progression **(Ma et al., 2018).** The anti-fibrotic action of pirfenidone may be due to inhibition of the production of reactive oxygen species, pro-fibrotic cytokines, like transforming growth factor-beta (TGF-beta), and inflammatory cytokines, like tumor necrosis factor-alpha (TNF-alpha). TGF-beta facilitates fibrotic processes in the lungs by inducing proliferation of macrophages and fibroblasts through platelet-derived growth factor (PGDF) expression, is considered the main mechanism of anti-fibrotic activity for pirfenidone. Pirfenidone also, has little immunosuppressive activity **(Takeda et al., 2014).**

Statins, 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG CoA) reductase inhibitors, are commonly prescribed as a lipid lowering medication. Moreover, statins exhibit an antifibrotic effect and multiple pleiotropic secondary effects both related and unrelated to their lipid-lowering effect **(Janicko et al., 2016)**.

Rosuvastatin, the statin with the longest half-life, exhibits greater efficacy in dyslipidemia treatment due to its low lipophilicity, greater binding with HMG-CoA reductase enzyme, higher hepatocyte selectivity, minimal metabolism and low tendency for hepatic cytochrome P450 (CYP) drug interactions as compared with other statins **(Khan et al., 2019).**

Bleomycin is a well-known anti-cancer drug. The major limiting factor for its use in cancer therapy is the possible development of varying degrees of pulmonary fibrosis in about 10 % of patients treated with bleomycin. Two FDA-approved oral medications have similar proven efficacy in slowing disease progression, but neither improves quality of life. Both are associated with substantial side effects e.g., pirfenidone, and Nintedanib. Additional new therapies are being investigated and are in phase I, II, and III trials.The combination of prednisone, azathioprine, and N-acetylcysteine was associated with increased mortality in a randomized clinical trial and is contraindicated **(Dempsey et al., 2019).**

TGF-β1 belongs to TGF-β superfamily. They control differentiation of cell and tissue physiology in eukaryotes. Over-expression of TGF-β1 in transgenetic mice induces and promotes tissue fibrosis **(Trendelenburg, 2020).** TGF- β1 is

a pro-fibrogenic cytokine that can directly induce differentiation of fibroblasts into myofibroblasts **(Walton et al., 2017).** So that, the aim of the work is to test the possible anti-fibrotic effect of rosuvastatin in comparison to well-established anti-fibrotic effect of pirfenidone for treatment of bleomycin-induced pulmonary fibrosis.

Materials and Method:

Chemicals: Bleomycin (Bleocel®) was obtained from CELON LABS Company in the form of vials containing lyophilized powder, 15 Units. It was dissolved in isotonic saline solution. Pirfenidone (Pirfenex®) was obtained from Cipla Company in the form of 200 mg tablets. It was dissolved in 0.5% carboxymethylcellulose (CMC) solution (vehicle). Rosuvastatin (Crestor®) was obtained from AstraZeneca Company in the form of 10 mg tablets. It was dissolved in *0.5% carboxymethylcellulose (CMC)* solution (vehicle).

Animals: Thirty-two (32) male, C57BL/6 mice (8–12 weeks; 20–22 g) were purchased from the Medical Experimental Research Center (MERC) (Faculty of Medicine, Mansoura, Egypt). Mice were randomly divided into 4 equal groups (n = 8/group), maintained under specific pathogen-free conditions and acclimated for 1 week at room temperature (22±2℃) in a 12-h light/dark cycle before the experiments, with food and water provided ad libitum. The local animal ethics committee approved all this experiment procedures.

Experimental design: Mice were randomly divided into **4 groups** (n=8 per group):

Group 1; Negative control group; received sterile phosphate-buffered saline (PBS) in 50 μL volume, injected intratracheally once at day 0.

Group 2; *Bleomycin-induced pulmonary fibrosis* (Positive control group); received bleomycin (2U/Kg), dissolved in 0.9% sterile normal saline, in 50 μL volume, injected intratracheally once at day 0 **(Liu et al., 2017a).**

Group 3; *Bleomycin-induced pulmonary fibrosis* group treated with ***Pirfenidone*** (300 mg/kg/day) via oral gavage once daily, starting from "the 10th day" to "the 21st day" post-bleomycin **(Liu et al., 2017b).**

Group 4; *Bleomycin-induced pulmonary fibrosis* group treated with ***Rosuvastatin*** (10 mg/kg/day) via oral gavage once daily, starting from "the 10th day" to "the 21st day" post-bleomycin **(Sikder et al., 2020).** At the end of the experiment, all mice were sacrificed under deep anesthesia.

Biochemical studies: Body Weights were measured at the start, every other day and at the end of the experiment. At "Day 21", mice were sacrificed and lungs were weighted for lung indices [lung coefficient ratio = lung weight (mg) / body weight (g)] which assess the severity of lung injury. The right lung lobes were homogenized for *TGF-β1 concentration* assay, using a mouse TGF-β1 enzyme -linked immunosorbent assay (ELISA) kits (Cat. No. E0660Mo) were brought from Shanghai Crystal Day Biotech Co., Ltd., China.

Histopathological studies: The left lung lobes were harvested for Histopathological examinations by Hematoxylin & Eosin stain and by Masson's Trichrome stainto detect the fibrosis.

Statistical Analysis

Analysis of data was done using Statistical package for social science (SPSS) program (version 24). Firstly, Shapiro test was done to test the normality of data. Continuous variables were introduced as mean±SD (standard deviation) for parametric data. ANOVA test was done to compare more than two means. Qualitative data were described using number and percent. Association between categorical variables was tested using Monte Carlo test. P values of ≤0.05 were considered statistically significant in all analyses.

Results

Effects of Pirfenidone and Rosuvastatin on body weight, lung weight and lung index:

1. **Body weight:**

As shown in table (1), induction of pulmonary fibrosis by bleomycin (BLM) induced a significant (P<0.001) decrease in body weight at day 21 (**19**.62±3.20g) as compared to control normal group (**27**.25±1.39g). Administration of pirfenidone, in dose of (300 mg/kg/d), to mice with BLM-induced pulmonary fibrosis, showed significant increase in body weight at day 21 (**22**.75±2.43g) as compared to non-treated BLM control group (**19**.62±3.20g). Administration of rosuvastatin, in dose of (10 mg/kg/d), to mice with BLM-induced pulmonary fibrosis, showed significant increase in body weight at day 21 (**22**.87±1.88g) as compared to non-treated BLM control group (**19**.62±3.20g).

2. **Lung weight:**

As shown in table (1), induction of pulmonary fibrosis by BLM induced a significant (P<0.001) increase in lung weight (**0.25**±0.01g) as compared to control normal group (**0.18**±0.04g). Administration of pirfenidone, in dose of (300 mg/kg/d), to mice with BLM-induced pulmonary fibrosis, significantly (P<0.001) reduced lung weight (0**.18**±0.02g) as compared to non-treated BLM control group (**0.25**±0.01g). Administration of rosuvastatin, in dose of (10 mg/kg/d), to mice with BLM-induced pulmonary fibrosis, significantly (P<0.001) reduced lung weight (**0.17**±0.01g) as compared to non-treated BLM control group (**0.25**±0.01g).

It is noted that mice treated by either pirfenidone (300 mg/kg/d) or rosuvastatin (10 mg/kg/d) were able to reach nearly the control value (**0.18**±0.04g).

3. **Lung index:**

As shown in table (1), induction of pulmonary fibrosis by BLM induced a significant (P<0.001) increase in lung index (**12**.61±2.28) as compared to **negative control normal group** (**6**.53±1.02). Administration of **pirfenidone**, in dose of (300 mg/kg/d), to mice with BLM-induced pulmonary fibrosis, significantly (P<0.001) reduced lung index (**7**.93±1.11) as compared to non-treated BLM control group (**12**.61±2.28). Administration of **rosuvastatin**, in dose of (10 mg/kg/d), to mice with BLM-induced pulmonary fibrosis, significantly (P<0.001) reduced lung index (**7**.33±0.35) as compared to non-treated BLM control group (**12**.61±2.28).

It is noted that mice treated by either pirfenidone (300 mg/kg/d) or rosuvastatin (10 mg/kg/d) were able to reach nearly the control value (**6**.53±1.02).

Effects of Pirfenidone and Rosuvastatin on lung TGFβ1 concentration:

As shown in table (2), induction of pulmonary fibrosis by BLM induced a significant (P<0.001) increase in lung TGF-β1 (**1331**.9±359.7 ng/L) as compared to control normal group (**323**.03±49.8 ng/L). Administration of pirfenidone, in dose of (300 mg/kg/d), to mice with BLM-induced pulmonary fibrosis, significantly (P<0.001) reduced lung TGF-β1 (**818**.16±183.5 ng/L) as compared to non-treated BLM control group (**1331**.9±359.7 ng/L). Administration of rosuvastatin, in dose of (10 mg/kg/d), to mice with BLM-induced pulmonary fibrosis, significantly (P<0.001) reduced lung TGF-β1 (**528**.3±64.14ng/L) as compared to non-treated BLM control group (**1331**.9±359.7 ng/L).

Moreover, administration of rosuvastatin, in dose of (10 mg/kg/d) to mice with BLM-induced pulmonary fibrosis, significantly (P=0.009) reduced lung TGF-β1 (**528**.3±64.14ng/L) as compared to mice with BLM-induced pulmonary fibrosis treated with pirfenidone (**818**.16±183.5 ng/L).

Effects of Pirfenidone and Rosuvastatin on histopathological score (fibrosis grade):

Paraffin sections were prepared and stained with Hematoxylin & Eosin (HE) stain and Masson's trichrome stain (which aimed to ascertain the fibrosis). Sections stained with Masson's trichrome stain, were graded with "the modified Ashcroft's scoring", to determine the extent of fibrosis **(Hübner et al., 2008).** The modified Ashcroft's scoring (which has grades from 0 to 8) was refined and collected into no, mild, moderate and severe grades by the pathologist (Table 3).

Hematoxylin and Eosin (HE) and Masson's trichrome-stained sections of mice lung were examined microscopically at a power of magnification of 10× and 20×. HE stained-sections from **negative normal control group** showed normal lung morphologies with normal alveolar pattern; thin alveolar wall, intact alveolar structure, normal alveolar septum, and less inflammatory cells infiltration in the pulmonary mesenchyme **(Figure 1)**, which was confirmed by Masson trichrome staining of lung sections **(Figure 2)**. HE stained-sections from the **BLM-induced pulmonary fibrosis group**, showed distorted lung morphologies: destruction of the alveolar architecture, interstitial fibrosis of the alveolar wall, increase in alveolar septum width, collapsed or disappeared alveoli and increased inflammatory cells and fibroblasts infiltration **(Figure 3)**. Masson's trichrome staining showed that lung tissue had an abnormal collagen deposition and distorted lung morphologies compared with the control animals (P1<0.001), indicated by extensive blue staining in the lung tissue and septum. Alveoli were nearly obliterated with fibrous masses, suggesting a severe degree of fibrosis **(Figure 4)**.

Treating mice with BLM-induced pulmonary fibrosis, with **pirfenidone**, at dose of 300mg/kg/d, showed significant change in histopathological score of BLM-induced pulmonary fibrosis (P2< 0.001 as compared to non-treated BLM-induced pulmonary fibrosis group). HE stained-sections showed decreased damaged lung tissue structure with lessened alveolar thickening **(Figure 5).** Pirfenidone strongly inhibited collagen extent and intensity in the Masson's trichrome-stained sections, as shown by that the blue area was decreased. In those Masson's trichrome-stained sections, there were just fibrotic changes in the alveolar septa, with no

fibrotic masses, suggesting a mild degree of fibrosis **(Figure 6).** Also, treating mice with BLM-induced pulmonary fibrosis, with **rosuvastatin**, at dose of 10mg/kg/d, showed significant change in histopathological score of BLM-induced pulmonary fibrosis (P2<0.001 as compared to non-treated BLM-induced pulmonary fibrosis group). HE stained-sections showed nearly preserved lung tissue structure **(Figure 7).** Also, Masson's trichrome-stained sections showed decreased blue area, with no fibrotic masses, suggesting a mild degree of fibrosis **(Figure 8).**

Table (1): Effects of Pirfenidone *(300 mg/kg/d, orally)* and Rosuvastatin *(10 mg/kg/d, orally)* on *body weight (g), lung weight (g)* and *lung index* in Bleomycin-induced pulmonary fibrosis in mice:

Parameters	**-ve control group** No. = 8 Mean ± SD	**+ve control group (BLM-induced pulmonary fibrosis)** No. = 8 Mean ± SD	**Pirf. Group** No. = 8 Mean ± SD	**Rosu. Group** No. = 8 Mean ± SD
Body weight (g) at Day 0	**24**.25±1.39	**25**.00±1.31	**26**.62±2.44	**25**.37±1.68
Body weight (g) at Day 21	**27**.25±1.39*	**19**.62±3.20*$^{\neq}$	**22**.75±2.43*$^{\neq\$}$	**22**.87±1.88*$^{\neq\$}$
Lung weight (g)	**0.18**±0.04	**0.25**±0.01$^{\neq}$	**0.18**±0.02$^{\$}$	**0.17**±0.01$^{\$}$
Lung index	**6**.53±1.02	**12**.61±2.28$^{\neq}$	**7**.93±1.11$^{\$}$	**7**.33±0.35$^{\$}$

Data were analyzed by *ANOVA test* followed by *post hoc LSD analysis* for comparison between groups, and are presented as *means ± SD*.

Abbreviations: "-ve" Negative, "+ve" Positive, "Pirf." Pirfenidone, "Rosu" Rosuvastatin, "No." number, "SD" standard deviation. "BLM" Bleomycin.

*****: Significant body weight variance at day 21 *relative to* body weight variance at day 0 in each group separately.

$^{\neq}$: Significance *relative to* negative control group.

$^{\$}$: Significance *relative to* positive control group.

Table (2): Effects of Pirfenidone *(300 mg/kg/d, orally)* and Rosuvastatin *(10 mg/kg/d, orally)* on *TGF-β1 concentration (ng/L)* in lung homogenates in Bleomycin-induced pulmonary fibrosis in mice:

Parameter	**-ve control group** No. = 8 Mean ± SD	**+ve control group (BLM-induced pulmonary fibrosis)** No. = 8 Mean ± SD	**Pirf. Group** No. = 8 Mean ± SD	**Rosu. Group** No. = 8 Mean ± SD
TGF-*β1* (ng/L)	**323**.03±49.8	**1331**.9±359.7$^{\neq}$	**818**.16±183.5$^{\neq\$}$	**528**.3±64.14$^{\$€}$

Data were analyzed by *ANOVA test* followed by *post hoc LSD analysis* for comparison between groups and are presented as *means ± SD*.

Abbreviations: "-ve" Negative, "+ve" Positive, "Pirf." Pirfenidone, "Rosu" Rosuvastatin, "No." number, "SD" standard deviation. "BLM" Bleomycin.

$^{\neq}$: Significance *relative to* negative control group.

$^{\$}$: Significance *relative to* positive control group.

$^{€}$: Significance *relative to* pirfenidone group.

Table (3): Characterization of the refined modified Ashcroft's score:

Grades	
No fibrosis.	**Alveolar septa**: *Normal*, **Lung structure**: *Normal*.
Mild fibrosis	**Alveolar septa**: □ *Isolated* fibrotic alterations[*1]. *OR* □ Clearly fibrotic alterations[*2] with *knot-like formation*. OR □ *Contiguous fibrotic walls* nearly in whole microscopic field. **Lung structure**: Alveoli partly increased-in-size and dislocated, but *fibrotic masses aren't present*.
Moderate fibrosis	**Alveolar septa**: Variable. **Lung structure**: *fibrotic masses*: □ Single[*3]. OR □ Confluent[*4] (severely disrupted).
Severe fibrosis	**Alveolar septa**: mostly OR totally Non-existent. **Lung structure**: □ Large contiguous fibrotic masses[*5] (Lung architecture mostly not preserved). OR □ Alveoli *nearly obliterated with fibrous masses* but still up to five *air bubbles*. OR □ Microscopic field with *complete obliteration* with fibrotic masses.

***1 (Isolated fibrotic alterations)** = *septum ≤3× thicker than normal.*
***2 (Clearly fibrotic alterations)** = *septum >3× thicker than normal.*
***3 (Single fibrotic masses)** = *≤10% of the microscopic field.*
***4 (Confluent fibrotic masses)** = *>10% and ≤50% of the microscopic field.*
***5 (Large contiguous fibrotic masses)** = *>50% of the microscopic field.*

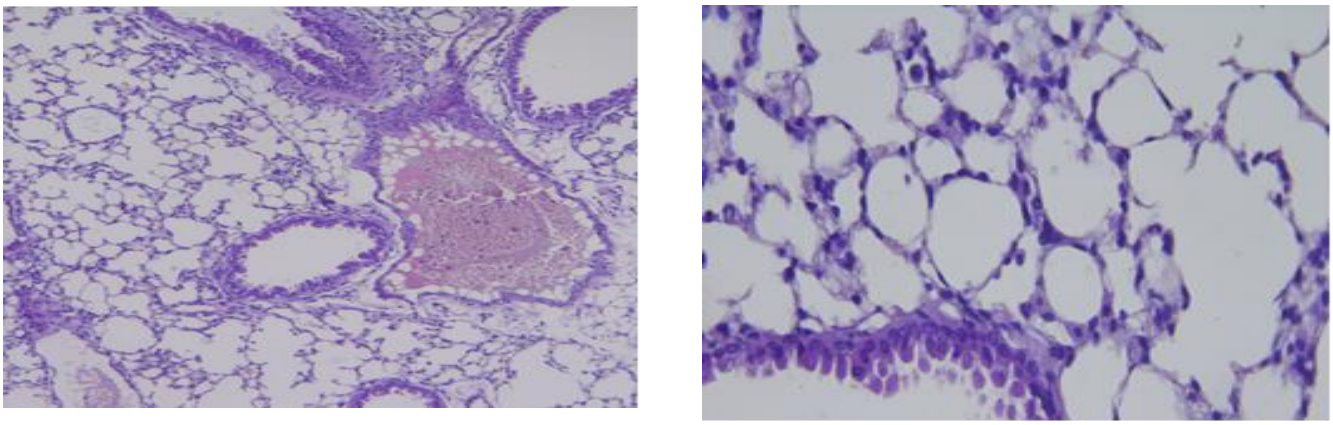

Figure (1): *Normal histological appearance of lung tissue sections (Hx. &E. stain, x20 & x40).*

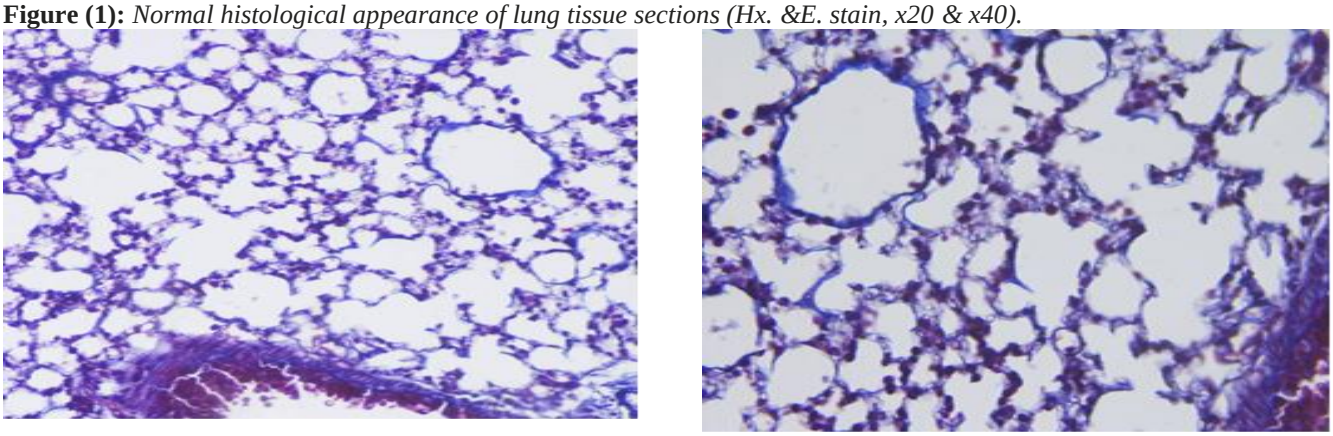

Figure (2): *Normal histological appearance of lung tissue sections, showing normal lung structure with no fibrosis (Masson's trichrome stain, x10 & x20).*

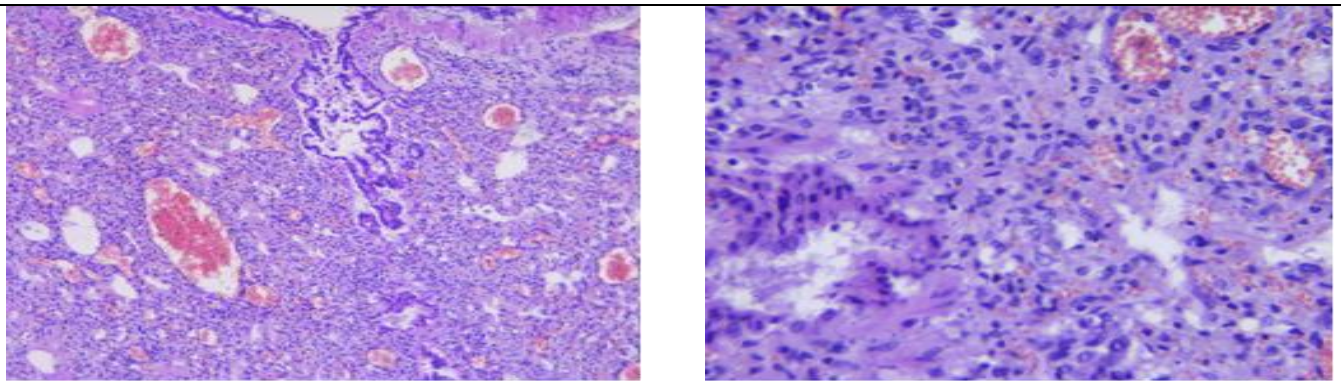

Figure (3): *Histopathological findings in lung tissue sections of non-treated BLM-induced pulmonary fibrosis group (Hx. & E. stain, x10 & x20).*

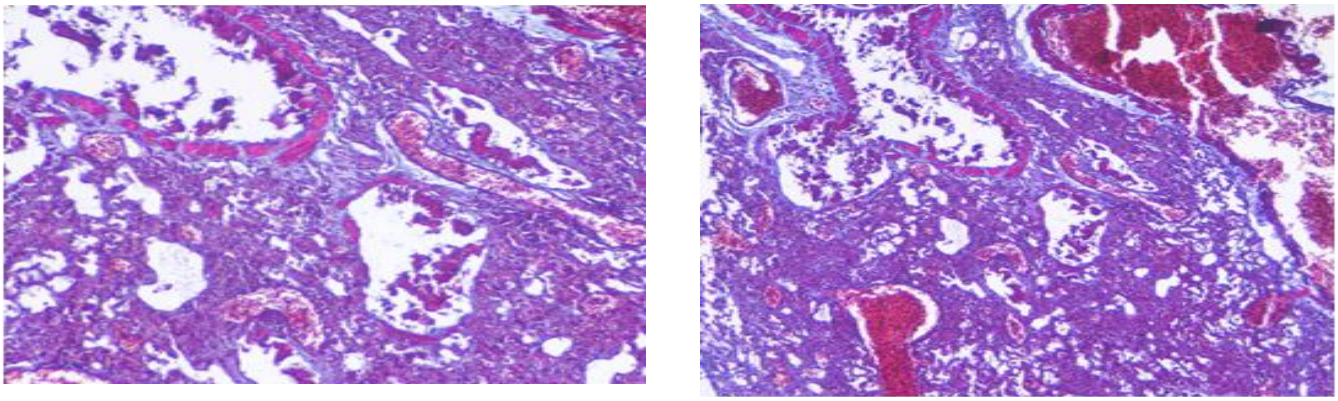

Figure (4): Histopathological findings in lung tissue sections of non-treated BLM-induced pulmonary fibrosis group, showing a severe fibrosis degree with nearly obliterated alveoli (Masson's trichrome stain, x10 & x20).

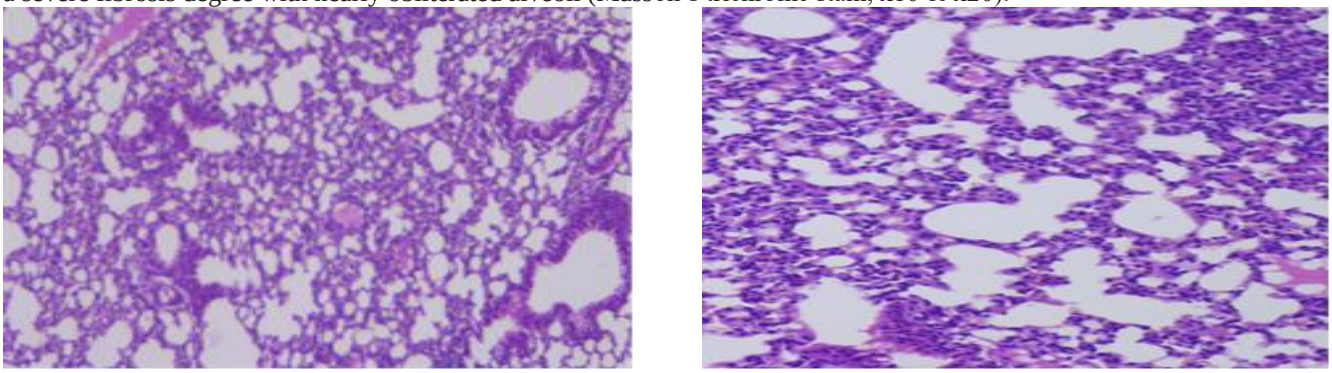

Figure (5): *Histopathological findings in lung tissue sections of BLM-induced pulmonary fibrosis group treated with pirfenidone 300/mg/kg/d (Hx. & E. stain, x10 & x20).*

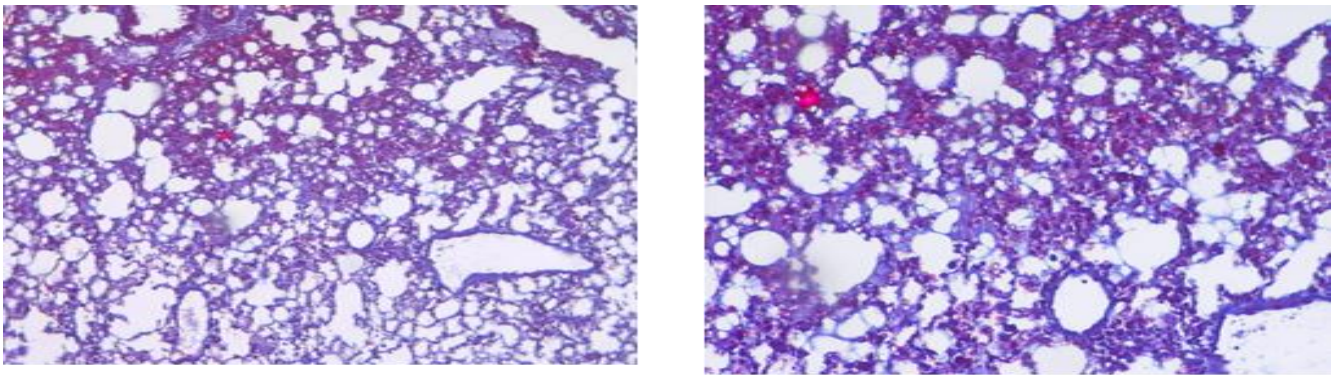

Figure (6): *Histopathological findings in lung tissue sections of BLM-induced pulmonary fibrosis group treated with pirfenidone 300/mg/kg/d, showing a mild fibrosis degree (Masson's trichrome stain, x10 & x20).*

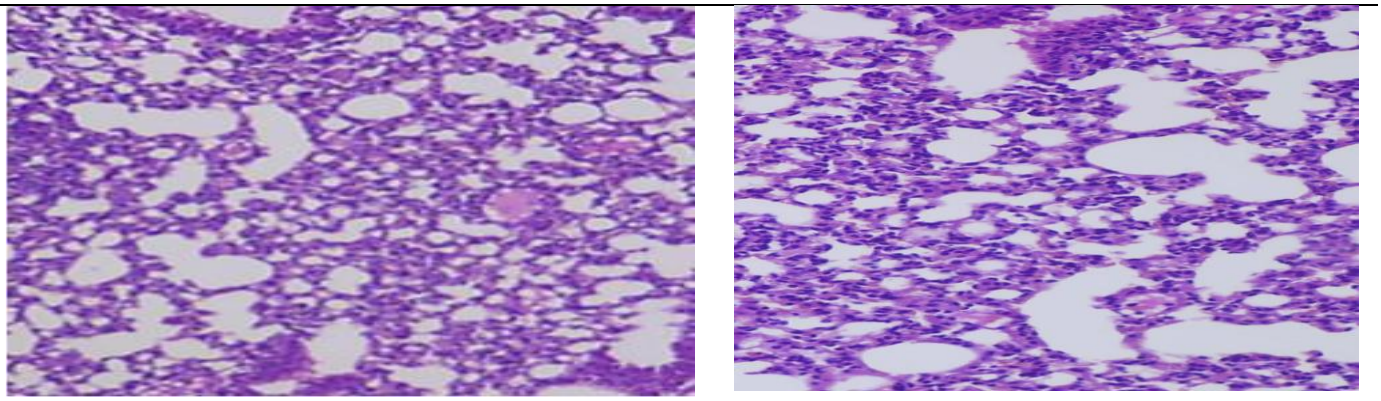

Figure (7): *Histopathological findings in lung tissue sections of BLM-induced pulmonary fibrosis group treated with rosuvastatin 10/mg/kg/d (Hx. & E. stain, x10 & x20).*

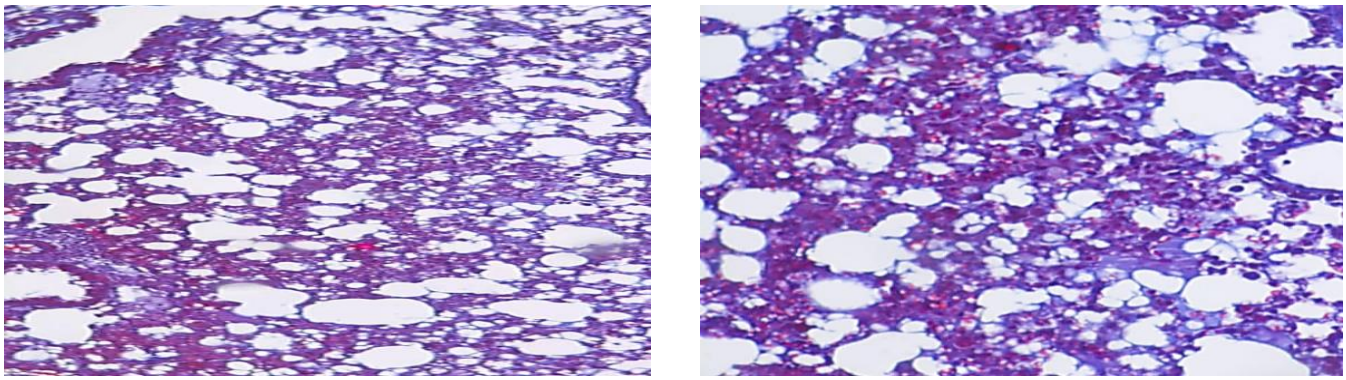

Figure (8): *Histopathological findings in lung tissue sections of BLM-induced pulmonary fibrosis group treated with rosuvastatin 10/mg/kg/d, showing a mild fibrosis degree (Masson's trichrome stain, x10 & x20).*

Discussion

IPF is a chronic and progressive ILD characterized by alveolar epithelial cells (AECs) injury, inflammatory cell infiltration, fibroblast proliferation and abnormal ECM deposition **(Liu and Shi, 2019)**.

Pirfenidone was approved by FDA for treatment of idiopathic pulmonary fibrosis in 2014. During randomized, double-blind, placebo-controlled trials, pirfenidone significantly reduced decline in forced vital capacity (FVC). But mortality was not significantly affected by pirfenidone across clinical trials and available follow-up periods. Liver enzyme elevations and drug-induced liver injury have been associated with pirfenidone; therefore, liver function monitoring is necessary during therapy **(Esbriet, 2022).**

Lung transplantation was the only effective treatment for IPF. FDA approved pirfenidone and nintedanib as anti-fibrotic agents for treatment of IPF. Although these drugs have improved the clinical management of IPF greatly, both drugs only slow lung function decline and can't halt or regress disease progression **(Ma et al., 2018)**. However, lung transplantation has many limitations attributable to organ shortages and complications associated with long-term immunosuppression. Therefore, the development of effective therapies to reduce or reverse pulmonary fibrosis is important clinically for reducing the morbidity and mortality associated

with IPF and the need for lung transplantation **(Zhu et al., 2013).**

In the current study, mice which received only bleomycin by single intra-tracheal injection, developed pulmonary fibrosis, which was evident from the significant decrease in final body weight with significant increase in lung weight, lung index and lung TGF-β1, compared to negative control group. Also, lung sections of these mice showed significant increase in the histopathological fibrosis (severe degree).

This is in line with the study of **Zhu et al. (2013),** who noted a significant decrease in the final body weight with a simulatenous significant increase in lung weight and indices in the bleomycin-treated animals. Also, **Guo et al. (2019),** stated that TGF-β1 is up-regulated in lung tissues of both bleomycin-treated mice and IPF patients and also noted final body weight loss with increased collagen accumulation in the Masson's trichrome stained sections in bleomycin group.

Pathologically; Alveolar Epithelial Cells (AECs) are permanently replaced by myofibroblasts, which are the chief generator of ECM proteins, like collagen and fibronectin. The profibrotic marker TGF-β is the best characterized activator of myofibroblast generation. Overexpression of active TGF-β1 induces prolonged and severe interstitial lung fibrosis in rats while deletion of TGF-β receptor from either fibroblast or epithelial cells protects mice from bleomycin-induced pulmonary fibrosis **(Guo et al., 2019).**

These effects of bleomycin on lung can be also explained by that bleomycin chelates metal ions forming a "pseudo-enzyme", then the formed pseudo-enzyme reacts with oxygen, resulting in production of DNA-cleaving reactive oxygen species (ROS) with the induction of oxidative stress. This causes breaks in one or both strands of DNA in AECs, resulting in disruption of cell cycle leading to cell death **(Jana et al., 2019).** In addition, bleomycin hydrolase, a bleomycin-inactivating enzyme, mainly affects drug effects on a tissue-specific basis. Lungs maintain low levels of this enzyme; therefore, they are susceptible to bleomycin-induced injury **(Moeller et al., 2008).**

Treating mice with pirfenidone 300mg/kg/d showed significant increase in final body weight with significant decrease in lung weight and lung index, significant decrease in lung TGF-β1, and significant decrease in the histopathological fibrosis. These findings are consistent with **Song et al. (2018),** who observed that pirfenidone alleviated the fibrosis degree in the lungs of mice with pulmonary fibrosis suggesting that pirfenidone can relieve the pathological progression of IPF.

This can be explained by affecting TGF-β1 as TGF-β signaling is linked to almost every form of fibrosis. Pirfenidone can downregulate TGF-β1 as well as a plethora of downstream TGF-β1-associated signaling mechanisms. Pirfenidone suppresses TGF-β-mediated fibroblast proliferation and differentiation into myofibroblasts, the chief generator of ECM proteins, like collagen and fibronectin; by attenuating TGF-β1/SMAD3-induced signaling. Also, it inhibits gene and protein expression of TGF-β1-induced mediators such as fibronectin and collagen, which are the main ECM proteins involved in the fibrotic process. Also, it reduces TGF-β1-induced α-smooth muscle actin (α-SMA) expression, a factor important for fibroblast to

myofibroblast transition **(Ma et al., 2018).** Moreover, pirfenidone, the TGF-β inhibitor, significantly suppresses the protein levels of Smad 2/3, p-Smad 2/3, and α-SMA in mice model of choroidal neovascular fibrosis **(Gao et al., 2021).**

Treating mice with rosuvastatin 10mg/kg/d showed significant increase in final body weight, significant decrease in lung weight and lung index, significant decrease in lung TGF-β1, much more significant than pirfenidone, and significant decrease in the histopathological fibrosis. These results are consistent with **Okada et al. (2013),** who noted that rosuvastatin down-regulated TGF-βmRNA level in a rat model of high-fat and high-cholesterol diet induced-liver fibrosis**.** Also, these results are consistent with **Boutari et al. (2019),** who mentioned that rosuvastatin improved both hepatic steatosis and fibrosis, up to that the percentage of patients with advanced fibrosis decreased from 13.6% to 4.6%.

However, there were studies which documented that statins are not beneficial for survival in IPF patients **(Alexeeff et al., 2007).** Also, a case study showed that one of the side effects of statins could be pulmonary interstitial disease **(Fernandez et al., 2008).** However, statins counteract the pulmonary complications of many drugs **(Choudhury et al., 2015)**.

Conclusion

Rosuvastatin attenuates the histological changes in pulmonary fibrosis and reduces the levels of TGF-β induced by bleomycin in mice. Therefore, it is concluded that, rosuvastatin can be a promising drug for IPF treatment due to its anti-fibrotic effect and safety profile. Thus, the pleiotropic actions of rosuvastatin might be applied clinically for prevention or treatment of bleomycin-induced pulmonary fibrosis. Combination of both pirfenidone and rosuvastatin for treatment of IPF need further studies. Moreover, more studies are recommended to search for the particular pathway of this anti-fibrotic effect of rosuvastatin in IPF.

Conflict of interests

The authors hereby declare that there is no conflict of interest as regards the publication of this paper.

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